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Nutritive value, antioxidant activity and phenolic compounds profile of brewer's spent yeast extract

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ABSTRACT

Spent yeast is the second major by-product from brewing. Mechanical disruption of yeast cell wall can be used to obtain β-glucan rich ingredients and separate inner yeast content, both with potential applications as food and nutraceutical ingredients. In this work, the nutritional composition, including minerals and B-complex vitamins, together with the antioxidant activity and phenolic compounds profile of yeast extract, prepared by mechanic disruption of brewer's spent yeast and removal of yeast cell wall, was investigated. The lyophilised yeast extract presented 64% of proteins with high proportion of essential amino acids and 4% of RNA; macrominerals (Na, K, Ca, Mg), trace elements such as Zn (11.9 mg/100 g dw), Fe (1.76 mg/100 g dw), and Mn (0.564 mg/100 g dw), and vitamins B3 (77.2 mg/100 mg dw), B6 (55.1 mg/100 g dw) and B9 (3.01 mg/100 g dw). Two phenolic compounds were quantified as free forms, gallic acid and (±) catechin, whereas other bounded phenolic compounds were also quantified. The nutrients content, antioxidant properties and phenolic composition of the lyophilised brewer's spent yeast extract indicates that it can be an interesting food or nutraceutical ingredient. Thus, its recovery will be beneficial in terms of sustainability and environmental impact.

1. Introduction

Waste prevention is a priority of the food and drink industry, however, full by-products reuse is still an unresolved problem. At present, the exploitation of food industry by-products is highly encouraged by the European Union legislation. This valorisation can be achieved through the extraction and characterization of high-value nutrients that can be reused as functional ingredients, which is beneficial in terms of sustainability and environmental impact (Baiano, 2014).

Brewing spent yeast is the second major by-product from the brewing industry. It is low in calories, fat and carbohydrates, however, it can be a valuable source of cheap fibre, mainly β-glucans (Liu et al., 2008; Aimanianda et al., 2009; Petravić-Tominac et al., 2011; Martins et al., 2015), nucleotides (Vieira et al., 2013), vitamins and minerals (Ferreira et al., 2010).

In the brewing process, serial repitching of *Saccharomyces* biomass is usual; thus, yeast is reused four to six times before its disposal (Vieira et al., 2012). Yeast presents adaptive response to oxidative stress similar to that of human cells, consequently vitamins, namely B6 and B12, and minerals (enzyme co-factors), such as zinc, copper and manganese can accumulate in yeast (Gaspar et al., 2008). Moreover, *Saccharomyces* adsorb phenolic compounds from exterior medium, increasing its antioxidant activity and phenolic compounds content (Rizzo et al., 2006). Although these compounds exhibit biological activities such as prevention of age-related diseases, inhibition of cancer cell proliferation and enhancement of immune response (Hassan, 2011; Jung et al., 2011; Bayarjargal et al., 2011), until now efforts to recover bioactive compounds from brewer's spent yeast are scarce.

Moreover, the separation of yeast compounds for use in food applications requires efficient means of disrupting cell walls and separating the products of interest. Mechanical processes can be used to separate yeast cell-wall β-glucans, which are known for lowering cholesterol and triacylglycerols in blood, enhancing the immune system and the anti-inflammatory activity, stimulating the skin cell response to combat free radicals and delaying aging

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process. The European Food Safety Authority (EFSA) has already approved the use of *Saccharomyces* β-glucans – referred to as "yeast beta-glucans" – as a new food ingredient and suggests a use ranging between 50 and 200 mg per serving (EFSA, 2011). However, new applications for the inner cell content are needed to make the process of β-glucans' separation profitable.

Attempts to reuse the brewer's spent yeast in biotechnological processes include production of flavour enhancers (In et al., 2005; Vieira et al., 2013). In a previous work, mechanical rupture of cell wall, using glass beads, to separate cell wall constituents and produce a yeast extract rich in nucleotides that can be used as flavour enhancers was described (Vieira et al., 2013). However, the full characterization of this extract is of major relevance to find new applications for brewing spent yeast. Thus, the goal of this work was to study the nutritional composition, the minerals and B-complex vitamins content, the antioxidant activity and the phenolic profile of the brewing spent yeast extract obtained by mechanical disruption, removal of cell wall and lyophilisation, to assess its potential interest as an ingredient in food and nutraceutical industries.

2. Material and methods

2.1. Reagents and standards

HPLC grade solvents were from Merck (Darmstadt, Germany). Iron (III) chloride hexahydrate; 2,4,6-tripyridyl-s-triazine (TPTZ); 2,2-diphenyl-1-picrylhydrazyl (DPPH); Trolox; potassium ferricyanide and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ultrapure water was obtained from a Seralpur Pro 90 CN water purification. For mineral analysis, all solutions were prepared using polypropylene laboratory ware: pipette tips (VWR, Radnor, PA), volumetric flasks (Kartell, Milan, Italy) and centrifuge tubes (TRP, Trasadingen, Switzerland). High purity HNO_3 (≥69% w/w, TraceSELECT® Ultra, from Fluka, L'Isle d'Abeau Chesnes, France) and H_2O_2 (30% v/v, TraceSELECT®; Fluka, Seelze, Germany) were used as received. Mineral standard solutions were prepared from AccuTrace™ (AccuStandard®, New Haven, CT, USA) 10 μg/mL multi-element ICP-MS standards. Single-element standard solutions of Na, K, Ca, Mg and Fe, water-soluble vitamins and polyphenol standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Samples

Six different batches of spent brewer's yeast biomasses from *Saccharomyces pastorianus*, collected between January and March of 2015 and with 3, 4, 5 and 6 repitchings in the brewing fermentation step, were kindly supplied as slurry by Unicer – Bebidas de Portugal, S.A. (S. Mamede de Infesta, Portugal). Biomasses were transported to the laboratory under refrigerated conditions, protected from light and stored at 4 °C until extract preparation (1 day maximum).

2.3. Brewer's spent yeast extract preparation

The debittering process and the mechanical disruption procedure of the yeast cells were performed as described by Vieira et al. (2013). All steps were carried out under refrigerated conditions to minimize autolysis. Yeast cell wall was removed by centrifugation and the resulting clear supernatants (extracts of the inner yeast content) obtained from the six yeast biomasses were freeze-dried and stored at −20°.

2.4. Proximate composition

The composition of brewer's spent yeast extracts were expressed as% in a dry basis (% dw). Moisture content was determined at 105 °C until constant weight. Total nitrogen was analysed by the Kjeldahl method (AOAC, 2000) and protein content was estimated using factor conversion of 6.25; free α-amino nitrogen was measured by the ninhydrin method using glycine as standard (Schmitt and Budde, 2012). Total lipid and ash content were determined according to AOAC methods (2000). Total carbohydrate was determined by difference. RNA was extracted according to Liwarska-Bizukojc and Ledakowicz (2001) method; the concentration of RNA was determined based on Herbert et al. (1971) method.

2.5. Amino acid composition and chemical score

Brewer's spent yeast extracts were submitted to protein hydrolysis with 6 M HCl at 110 °C for 24 h and further derivatization was conducted according to the method validated in our laboratory (Pérez-Palacios et al., 2014). All amino acids except tryptophan were separated by gas chromatography-mass spectrometry (GC/MS) and the relative amino acid composition was expressed as g/100 g of protein. The parameters used for estimating the nutritive value of the brewer's spent yeast extract protein fraction were: (1) essential amino acid (EAA) index, calculated considering essential amino acids in the standard protein as described by FAO/WHO (1990); chemical score and protein efficiency ratio (PER), calculated from the equation developed by Lee et al. (1978).

Chemical score =% EAA in yeast extract/% EAA in standard protein

$$PER = -1.816 + 0.435[Met] + 0.780[Leu] + 0.211[His] - 0.944[Tyr]$$

2.6. Analyses of minerals

2.6.1. Sample preparation

Microwave-assisted acid digestion was performed in PTFE vessels using 300 mg of sample, 4 mL of 69% (w/w) HNO_3 and 1 mL of 30% (v/v) H_2O_2 and the following microwave heating program: 250 W for 1 min, 0 W for 2 min, 250 W for 5 min, 400 W for 5 min, and 600 W for 5 min. After digestion, vessels content was transferred to 25 mL volumetric flasks and the volume was made-up with ultrapure water. Solutions were then analysed by inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS).

2.6.2. Inductively coupled plasma mass spectrometry analysis

ICP-MS analyses were carried out under the following instrumental conditions: argon flow rate 14 L/min; auxiliary argon flow rate 0.8 L/min; nebulizer flow rate 0.95 L/min; RF power 1550 W. The elemental isotopes (m/z ratios) ^{52}Cr, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{65}Cu, ^{66}Zn, ^{82}Se and ^{95}Mo were monitored for analytical determinations; ^{45}Sc, ^{89}Y, ^{115}In and ^{159}Tb were used as internal standards. The instrument was tuned daily for maximum signal sensitivity and stability as well as for low oxides and double charged species formation using the Tune B iCAP Q solution (Thermo Fisher Scientific; 1 μg/L of Ba, Bi, Ce, Co, In, Li and U in 2% HNO_3 + 0.5% HCl). The internal standard solution was prepared by appropriate dilution of the corresponding AccuStandard® (New Haven, CT, USA) solution (ICP-MS-200.8-IS-1: 100 μg/mL of Sc, Y, In, Tb and Bi). Calibration standards were prepared from a SCP Science (Baie-d'Urfé, Quebec, Canada) 100 μg/mL multi-element ICP-MS standard solution (*Plasma*CAL SCP-33-MS). The detection

limits were calculated as the concentration corresponding to 3 standard deviations of 10 replicate integrations of the blank (HNO_3 2% v/v). Certified reference material BCR 679–cabbage (Sigma-Aldrich, St Louis, MO, USA) was used for evaluation of accuracy and repeatability.

2.6.3. Atomic absorption spectrometry analysis

Determination of Na, K, Ca and Mg was performed using a Perkin Elmer (Überlingen, Germany) 3100 flame (air-acetylene) atomic absorption spectrometer instrument.

2.7. Analysis of B-complex vitamins

2.7.1. Extraction of B-complex vitamins

Extraction of vitamins from brewer's spent yeast extracts was performed according to Parlog et al. (2008). The lyophilized extract (1 g) was homogenized with 10 mL of mobile phase A (50 mM ammonium acetate/methanol, 99:1), sonicated for 30 min at 27 ± 3 °C in ultrasonic water bath (FungiLab SA, Barcelona, Spain), centrifuged and then filtered through a 0.22 μm membrane, prior to HPLC injection.

2.7.2. Preparation of B-complex vitamins standard solution

The aqueous stock solutions of each vitamin were prepared every week and stored in the refrigerator in amber-glass bottles to protect vitamins from light-induced oxidation. Individual standard stock solutions of vitamin B2 (riboflavin), B3 (nicotinic acid), B6 (pyridoxine), and B12 (cyanocobalamin) were prepared by dissolving 10 mg of each compound in 10 mL of ultrapure water containing 0.01% of trifluoracetic acid as described by Grotzkyj et al. (2012). Standard stock solution of folic acid (B9) was prepared by dissolving 10 mg of the compound in 100 mL of pure water containing 4 mL of 1 M NaOH, as described by Ciulu et al. (2011). Working standard solutions were prepared fresh daily and kept protected from light. The calibrations curves were made by running 6 different standard solutions (6 concentration levels) of each vitamin in the HPLC system.

2.7.3. Chromatographic conditions for separation of B complex vitamins

High Performance Liquid Chromatography (HPLC) analysis was carried out according to the procedure describe by Parlog et al. (2008). The injection volume was 30 μL and all samples were run in triplicate. Photodiode array detection (PDA) was used to perform spectral scans over the range 230–360 nm and quantification was conducted at 260 nm for riboflavin, nicotinic acid and pyridoxine, and at 280 nm for folic acid and cyanocobalamin. Peak identification and purity were investigated by comparing the UV spectra of each individual compound when analysed in mixtures or in single compound standard solutions. Linearity was performed by preparing solutions of working standards mixture at six concentration levels. The limit of detection (LOD) and limit of quantification (LOQ) was based on signal-to-noise ratio. A signal-to-noise ratio of 3:1 was considered acceptable for estimating the LOD, whereas the LOQ was based on signal-to-noise ratio of at least 10:1. Precision of the method was evaluated by estimating the repeatability and intermediate precision of the analytical method. The repeatability was studied by running 10 consecutive replications of the same sample and calculating the% RSD for peaks area. The intermediate precision was calculated as the% RSD of peaks area across three consecutive analytical days. Analysis was carried out using an analytical HPLC system (Jasco, Tokyo, Japan) equipped with a quaternary low pressure gradient HPLC pump (Jasco PU-1580), a degasification unit (Jasco DG-1580-53 3-line degasser), an autosampler (Jasco AS-2057-PLUS), a MD-910 multiwavelengh detector (Jasco) and a 7125 Rheodyne injection valve (California, USA). The column was a Chrompack P 300 RP (polystyrenedivinylbenzene copolymer, 8 μm, 300 Å, 150 × 4.6 mm i.d.) (Chrompack, Middleburg, The Netherlands). Data acquisition was accomplished using Borwin Controller software, version 1.50 (JMBS Developments, Le Fontanil, France).

2.8. Antioxidant activity of yeast extracts

2.8.1. Ferric reducing antioxidant potential assay

The measurement of the Ferric Reducing Antioxidant Potential Assay (FRAP) was done according to Jansen and Ruskovska (2013). Trolox was used as standard at 0.0025-0.125 mg/ mL to generate a calibration curve and results (mean values ± SD) were expressed as milligrams of Trolox Equivalent per gram of dry weight extract (mg TE/100 g dw).

2.8.2. DPPH radical scavenging capacity

The DPPH (1,1-Diphenyl-1-Picrylhydrazyl) radical-scavenging capacity assay was performed as described by Herald et al. (2012). Trolox was used as standard at 0.0025-0.125 mg/ mL to generate a calibration curve and data was reported as means ± SD for three replications. Results (mean ± SD) were expressed as milligrams of Trolox Equivalent per gram of dry weight extract (mg TE/100 g dw).

2.8.3. Ferricyanide reducing power

The ferricyanide reducing power was determined as described by Almeida et al. (2011). Trolox was used as standard at 0.0025–0.125 mg/ mL to generate a calibration curve and results (mean ± SD) were expressed as milligrams of Trolox Equivalent per gram of dry weight extract (mg TE/100 g dw).

2.9. Extraction of phenolic compounds

The extraction of phenolic compounds was conducted as described by Khanam et al. (2012): 50 mg of lyophilized brewer's spent yeast extract were homogenized with 1 mL of mobile phase A for extraction of free phenolic compounds, whereas bounded phenolic compounds were extracted after alkaline hydrolysis: 50 mg of lyophilized brewer's spent yeast extract was treated with 1 mL of 2 M NaOH for 2 h at room temperature and constant agitation (250 rpm). After centrifugation at 5000 rpm for 5 min, the resultant extract was acidified to pH 2 using 6 M HCl and extracted three times with diethyl ether. The ether extracts were mixed, evaporated to the dryness under vacuum at 35 °C and finally dissolved in 1 mL of mobile phase A.

2.9.1. HPLC analysis of phenolic compounds

HPLC analysis was carried out according to the procedure described by Khanam et al. (2012) with some adjustments. The binary mobile phase consisted of 6% (v/v) glacial acetic acid in water (solvent A) and acetonitrile (solvent B), and was pumped at a flow rate of 0.7 mL/min for a total run time of 75 min, at temperature of 35 °C. A gradient program was used as follows: 0–3.5% B for 11 min, 3.5–5% B for 9 min, 5–10% B for 3 min, 10–13% B for 7 min, 13–15% B for 15 min, 15–30% B for 15 min, 30–50% B for 5 min, 50–100% B for 5 min and returning to the starting conditions for 5 min (0% B) before the next sample injection. The injection volume was 20 μL. Photodiode array detection (PDA) was used to perform spectral scans over the range 190–400 nm and quantification was conducted at 236 nm for (±)-catechin, (−)-epicatechin and rutin; at 260 nm for protocatechuic and vanillic acids; at 280 nm for gallic, syringic and cinnamic acids; at 320 nm for the derivatives of cinnamic acid (caffeic, *p*-coumaric, chlorogenic and ferulic acids) and at 350 nm for isoquercetin. Phenolic compounds identification was performed by comparison with retention times and spectra of standards as well as co-elution after fortification

with the standards and peak purity evaluation. Quantification was based on external standards calibration. The limit of detection (LOD), limit of detection quantification (LOQ) and precision were determined as described for B complex vitamins. The HPLC system was from Gilson (Villiers le Bel, France), consisting of two pumps (305 and 306), an 805 manometric module, a 811C dynamic mixer, an injection port with a 20 μL loop (Rheodyne, Rohnert Park, California, USA) and a photodiode array detector (Varian, Santa Clara, Califórnia, USA) controlled by a data processor software (Varian Santa Clara, Califórnia, USA). Chromatographic separation was achieved with a 150 × 4.6 mm, Spherisorb® ODS-2 80 Å (3 μm particle size) column from Waters (Milford, Massachusetts, USA).

3. Statistical analysis

All statistical analyses were performed using the software SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). Six independent experiments were always performed and analysed in duplicate (n = 12).

4. Results and discussion

4.1. Nutritional composition of brewer's spent yeast extracts

The proximate composition of freeze-dried brewer's spent yeast extracts obtained using a mechanic disruption process is shown in Table 1. Brewer's spent yeast extracts contained low moisture (7.7%), low fat content (1.32% dw) and high protein content (64.1% dw), which contributes to its stability during storage. Protein content was in agreement with the value reported by Caballero-Córdoba and Sgarbieri (2000), in which mechanic disruption using glass beads was also the process used for yeast extract production, although brewer's yeast suffered an alkaline treatment for debittering. The ash content (14.0%) and the total amino nitrogen (3.79%) were in agreement with the values reported by Saksinchai et al. (2001), in which a autolysis process (50 °C, 20 h) was adopted to produce the brewer's yeast extract. Also, the RNA content (4.00%) was in agreement with Vieira et al. (2013), confirming the potential use of this extract for flavour enhancers production.

4.2. Amino acid composition

The amino acid composition of brewer's spent yeast extracts and chemical scores are also presented in Table 1. Results indicated an amino acid profile rich in essential amino acids compared to the reference amino acid pattern recommended by FAO/WHO (1990) for adult humans. These results also agree with the amino acid profile obtained by Caballero-Córdoba and Sgarbieri (2000). Generally, S-amino acids (methionine and cysteine) are the limiting factor to the nutritive value of yeast protein. However, as reported in Table 1, S-amino acids were above the FAO/WHO reference. Essential amino acids account for about 40% of total amino acids, being in agreement with the reference value recommended by FAO/WHO (1990). The protein efficiency ratio (PER) was 2.39 and brewer's spent yeast extracts presented a high content of the flavor enhancer amino acids (glutamic acid, aspartic acid, glycine and alanine), accounting to 34% of total amino acids. The amino acid composition indicates that protein fraction of brewer's spent yeast extracts presents a good potential for

Table 1
Nutritional composition of brewer's spent yeast extract.

Proximate composition	(g/100 g dw)	Amino acids (g/100 g protein)		Reference Protein[b]	Chemical Score
Moisture (%)	7.70 ± 0.12	Alanine[d]	9.29 ± 0.31	–	
Ash	14.0 ± 0.2	Arginine[c]	6.00 ± 0.79	–	
Protein	64.1 ± 0.2	Aspartic acid[d]	5.98 ± 0.22	–	
α-amino nitrogen	3.79 ± 0.23	Cysteine	2.19 ± 0.01	–	
Fat	1.32 ± 0.04	Glutamic acid[d]	15.0 ± 0.39	–	
Carbohydrates [a]	12.9 ± 0.1	Asparagin	2.00 ± 0.01	–	
RNA	4.00 ± 0.16	Glutamine	3.13 ± 0.21	–	
		Glycine[d]	3.69 ± 0.60	–	
Macrominerals (mg/100 g dw)		Histidine[c]	11.9 ± 0.80	1.6	7.48
Sodium (Na)	1228 ± 22	Isoleucine[c]	3.23 ± 0.03	1.3	2.48
Potassium (K)	9148 ± 69	Leucine[c]	3.51 ± 0.20	1.9	1.85
Calcium (Ca)	27.1± 0.40	Lysine[c]	3.16 ± 0.23	1.6	1.98
Magnesium (Mg)	273 ± 2.31	Methionine[c]	2.28 ± 0.15	1.7	1.34
		Phenylalanine[c]	3.01 ± 0.01	–	
Trace elements (mg/100 g dw)		Proline	2.65 ± 0.03	–	
Chromium (Cr)	0.019 ± 0.000	Serine	4.60 ± 0.21	–	
Iron (Fe)	1.76 ± 0.03	Threonine[c]	2.60 ± 0.00	0.9	2.89
Manganese (Mn)	0.564 ± 0.013	Tyrosine	2.15 ± 0.00	–	
Cobalt (Co)	0.030 ± 0.001	Valine[c]	4.49 ± 0.08	1.3	3.45
Molybdenum (Mo)	0.003 ± 0.000	Triptophan	Nd	1.6	
Zinc (Zn)	11.9 ± 0.29				
Copper (Cu)	0.364 ± 0.001	TAA	90.9 ± 8.55		
Selenium (Se)	0.030 ± 0.000	EAA[c]	40.2 ± 4.57		
		FAA[d]	34.0 ± 3.04		
Vitamins (mg/100 g dw)		PER	2.39 ± 0.78		
Nicotinic acid (B3)	77.2 ± 1.1				
Pyridoxine (B6)	55.1 ± 2.5				
Folic acid (B9)	3.01 ± 0.02				
Riboflavin (B2)	Nq (0.329)				
Cyanocobalamin (B12)	Nq (0.256)				

Data are expressed as mean values ± standard deviation of six independent experiments, analysed in duplicate (n = 12).
Nq: not quantified, concentrations <LOQ (values represented in brackets in mg/100 g); Nd: not determined; Total amino acid (TAA); ; Protein efficiency ratio (PER).
[a] Calculated by subtraction of crude protein, total fat, moisture and ash contents from the total weight.
[b] Suggested profile of essential amino acid (EAA) requirements for adults, FAO/WHO (1990).
[c] Essential amino acid (EAA).
[d] Flavour amino acids (FAA).

applications in food and dietary supplement industries as a protein rich ingredient.

4.3. Minerals content

The macrominerals (Na, K, Ca, Mg) and trace elements (Cr, Fe, Mn, Cu, Co, Mo, Se, Zn) composition of brewer's spent yeast extracts is also presented in Table 1. Linear range, detection and quantification limits of inductively coupled plasma mass spectrometry and atomic absorption spectrometry methods were evaluated, additionally Certified Reference Material was analysed to assure the accuracy and repeatability of results. The analytical features of both methods are presented in Table 2. Due to the lack of information regarding the mineral composition of yeast extracts prepared by mechanic disruption using glass beads, as performed in this work, results were compared with the mineral profile of yeast extracts as reported by Alvim et al. (1999), in which a dehydrated yeast extract was prepared by spray dryer. The sodium content, 1228 mg/100 g dw, was lower than data reported by Alvim et al. (1999), 1475 mg/100 g. By the contrary, the potassium content (91.48 mg/100 g dw) was similar to the content reported by Alvim et al. (1999), 99 mg/100 g. Both minerals play an important role in the regulation of cell acid-base balance and water retention, and are essential for ribosomal protein synthesis. The calcium and magnesium contents were 27.11 mg/100 g dw and 273.6 mg/100 g dw, respectively. These elements helps in bone formation, muscle function, neurotransmission, cell division and blood coagulation and magnesium is important in the appropriate utilization of vitamins B and E and in maintaining fluid and electrolyte balance. According to the National Academy of Science (2011) the Dietary Reference Intakes (DRIs) of these elements for a young adult are 1500, 4700, 1000 and 420 mg, respectively for Na, K, Ca and Mg.

Concerning to trace elements, mean contents of 0.019 mg/100 g dw and 0.030 mg/100 g dw were found for Cr and Mo, respectively, whereas the content of Zn was 11.9 mg/100 g dw, Fe content was 1.755 mg/100 g dw, and Mn content was 0.564 mg/100 g dw. According to the National Academy of Science (2011), the Dietary Reference Intakes (DRIs) of these elements for a young adult are 0.025-0.035 mg for Cr, 0.045 mg for Mo, 8–11 mg for Zn, 8–18 mg for Fe, and 1.8–2.3 mg for Mn. The yeast extract can be used as supplement to fulfil these requirements.

4.4. B-complex vitamins content

The analytical features of the HPLC method used for quantification of B complex vitamins are summarized in Table 3. Only vitamins B3, B6 and B9 were quantified in the brewer's spent yeast extracts (Table 1). The mean content of vitamin B3 (77.2 mg/100 g dw) was significantly higher than the content reported by Pinto et al. (2013) (0.79 mg/100 g) for lyophilized brewer's yeast surplus obtained after alkaline treatment without removal of cell wall. Brewer's yeast is considered one of the best dietary sources of vitamin B3; this vitamin participates in several metabolic functions and also assists in antioxidant and detoxification functions. The mean content of vitamin B6 (pyridoxine) was 55.1 mg/100 g dw, which was significantly higher than the content reported by Pinto et al. (2013) for lyophilized brewer's yeast surplus, which was 9.99 mg/100 g. This vitamin plays a vital role in the function of several enzymes that catalyse essential chemical reactions in the human body, especially those involved in protein and amino acid metabolism. Regarding to vitamin B9 (folic acid), the mean content found (3.01 mg/100 g dw) was also higher than the values reported by Pinto et al. (2013) (0.25 mg/100 g). This vitamin has a central role in one-carbon metabolism; derivatives of folate act as co-factors, carrying one-carbon units for various reactions in the cell, such as synthesis of certain amino acids and nucleotides (Hjortmo et al., 2005). Riboflavin (vitamin B2) and cyanocobalamin (vitamin B12) were not detected (LOD of 0.099 μg/mL and 0.077 μg/mL, respectively) in brewer's spent yeast extracts. Pinto et al. (2013) quantified both vitamins (at 1.38 and 75.8 mg/100 g mean levels, respectively), but only in the lyophilised yeast without any treatment. These results indicate that the processes used to obtain the yeast extracts significantly influence their vitamin composition, but the removal of yeast wall concentrated the content of vitamins B3, B6 and B9.

4.5. Antioxidant activity

The mean values of antioxidant activity of yeast extracts evaluated by FRAP, DPPH and Ferricyanide Reducing Power were, respectively, 261 ± 14; 59.7 ± 2.5 and 127.6 ± 1.0 mg TE/100 g dw. Significant positive correlations were observed between results from FRAP versus DPPH ($R^2 = 0.972$) and versus Ferricyanide Reducing Power ($R^2 = 0.875$), and also between DPPH versus Reducing Power ($R^2 = 0.963$).

Table 2
Analytical features of inductively coupled plasma mass spectrometry and atomic absorption spectrometry methods evaluated by linear range, limit of detection (LOD), limit of quantification (LOQ), repeatability and analyses Certified Reference Material was analysed.

Element	Linear range[a] (μg/L)	LOD (mg/100 g)	LOQ (mg/100 g)	Certified value mg/100 g (mean ±uncertainty)	Determined value mg/100 g (mean ±SD)	Repeatability (% RSD; n = 10)
			FAAS			
Na	200–1000	0.105	0.318	–	–	–
K	400–2000	0.203	0.616	–	–	–
Ca	1000–5000	0.333	0.101	776.8 ± 65.5	795.2 ± 3.8	0.48
Mg	100–500	0.031	0.094	136.2 ± 12.7	137.2 ± 1.1	0.80
			ICP-MS			
Cr	1–100	0.00062	0.0019	0.06 ± 0.01	0.55 ± 0.08	14.5
Cu	1–100	0.00008	0.0002	2.89 ± 0. 12	2.86 ± 0.34	0.12
Co	1–100	0.000003	0.00001	–	–	–
Fe	1–100	0.00611	0.0185	5.50 ± 0.25	5.37 ± 0.08	1.49
Mn	1–100	0.00007	0.0002	1.33 ± 0.05	1.30 ± 0.01	0.76
Mo	1–100	0.00011	0.0003	1.48 ± 0.05	1.51 ± 0.03	1.99
Se	1–100	0.00093	0.0028	–	–	–
Zn	1–100	0.00047	0.0011	7.97 ± 0.27	8.04 ± 0.16	1.99

[a] Calibration curves were constructed in this linear range with $R^2 > 0.999$

Table 3
Analytical parameters for High Performance Liquid Chromatography-Diode Array detection (HPLC-PDA) quantification of B-complex vitamins.

Compound	UV band, nm (Max)	Regression equation[a]	R^2	Linear Range (μg/mL)	LOD[b] (mg/100 g)	LOQ[c] (mg/100 g)	Precision, peak area (% RSD)[d]	
							Intra-day	Inter-day
B3	221, (263)	$Y = 1.34E^{+07}X + 5.76E^{+05}$	0.9969	0.05–500	0.047	0.156	0.39	2.01
B6	(257), 289, 327	$Y = 1.98E^{+07}X - 2.88E^{+05}$	0.9981	0.05–500	0.032	0.105	0.74	1.74
B9	(282), 347	$Y = 3.62E^{+07}X + 4.14E^{+05}$	0.9996	0.05–100	0.017	0.058	1.12	2.58
B2	(257), 361	$Y = 6.35E^{+06}X + 9.09E^{+04}$	0.9998	0.01–100	0.099	0.329	0.66	3.24
B12	(271), 305, 361	$Y = 8.13E^{+06}X + 2.27E^{+06}$	0.9998	0.05–100	0.077	0.256	0.28	3.05

SD; standard deviation, LOD, limit of detection; LOQ; limit of quantification.
[a] *X* is the peak area and Y is the concentration (μg/mL) of the compound.
[b] LOD was calculated based on a signal-to-noise ratio of 3:1 (n = 10).
[c] LOQ was calculated based on a signal-to-noise ratio of 10:1 (n = 10).
[d] RSD (%) = ((standard deviation/mean) x 100) (n = 10).

4.6. Phenolic compounds

Thirteen phenolic compounds were separated by HPLC, the elution order was as follows: gallic acid, protocatechuic acid, (±)-catechin, p-coumaric acid, caffeic acid, vanillic acid, chlorogenic acid, (−)-epicatechin, syringic acid, ferulic acid, rutin, isoquercetin, cinnamic acid. Chromatographic peaks were numbered from 1 to 13 by elution order. The analytical features of the HPLC-PDA method used for quantification of phenolic compounds are summarized in Table 4. Yeast free and bound phenolic compounds were analysed. As shown in Table 5, only gallic acid and (±)-catechin were quantified in the free fraction, being the (±)-catechin the most representative compound (62%). In the bound fraction, six phenolic compounds were quantified, in the following order of abundance: cinnamic acid (1.24 mg/100 g dw), gallic acid (2.11 mg/100 g dw), ferrulic acid (9.22 mg/100 g dw), *p*-coumaric acid (10.3 mg/100 g dw), protocatechuic acid (13.1 mg/100 g dw) and (+)-catechin (24.6 mg/100 g dw), whereas the other phenolic compounds under study were not detected (Fig. 1). Four unknown peaks were observed in the chromatograms of yeast bond phenolic fraction, however, no similarities of UV spectra were found with the phenolic compounds under study and no co-elution was observed after fortification with the standards of caffeic acid, vanillic acid, chlorogenic acid, (−)-epicatechin, syringic acid, rutin, isoquercetin. The total phenolic compounds content in the free and bound fractions were, respectively, 55.5 mg/100 g dw and 60.6 mg/100 g dw. No information was found in literature concerning phenolic compounds content in brewer's spent yeast.

5. Conclusions

This study provides a detailed analysis of the nutritional composition, antioxidant activity and phenolic compounds profile of brewer's spent yeast extracts produced by mechanic disruption of spent brewer's yeast (*Saccharomyces pastorianus*) and removal of the cell walls (for separation of β-glucans). Results showed that the extracts from the inner content of yeast cells are a rich source of proteins containing essential amino acids, RNA, vitamins (B3, B6 and B9) and minerals. Higher contents were observed in comparison with other brewing yeast extracts described in the literature. Chromatographic analysis also showed that brewer's spent yeast extracts contains phenolic compounds in both the free and bounded forms: gallic acid, protocatechuic acid, (±)catechin, *p*-coumaric, ferulic and cinnamic acids were quantified. Additionally, brewer's spent yeast extracts present antioxidant activity, which makes this yeast extract a potential ingredient to be used in the formulation of functional foods and nutraceuticals. Moreover, since brewer's spent yeast extracts production complements the use of yeast cell wall for β-glucans and fibre obtaining, it makes the whole process much cost-effective.

Table 4
Analytical parameters for High Performance Liquid Chromatography-Diode Array detection (HPLC-PDA) quantification of phenolic compounds.

Compound	Peak number	t_R (min)	UV band, nm (Max)	Regression equation[a]	R^2	Linear Range (μg/mL)	LOD[b] (mg/100 g)	LOQ[c] (mg/100 g)	Precision, peak area (% RSD)[d]	
									Intra-day	Inter-day
Gallic acid	1	4.27	(271)	$Y = 1.15E^{+06}X - 1.01E^{+06}$	0.9964	0.01 – 300	0.070	0.232	0.25	1.38
Protocatechuic acid	2	4.68	240, (260), 294	$Y = 7.15E^{+05}X - 4.07E^{+06}$	0.9997	0.05 – 200	0.074	0.250	0.32	2.87
(±)-catechin	3	10.01	(236)	$Y = 2.16E^{+05}X - 2.43E^{+06}$	0.9982	0.05 – 300	0.028	0.096	0.65	1.54
p-coumaric acid	4	14.21	228, (309)	$Y = 3.99E^{+05}X - 1.90E^{+06}$	0.9996	0.05 – 200	0.082	0.274	1.01	2.54
Caffeic acid	5	15.10	239, (324)	$Y = 6.22E^{+05}X + 2.75E^{+06}$	0.9954	0.01 – 300	0.020	0.068	0.58	3.24
Vanillic acid	6	17.70	219, (260), 294	$Y = 8.89E^{+05}X - 4.68E^{+06}$	0.9995	0.05 – 200	0.048	0.160	0.89	3.05
Chlorogenic acid	7	20.40	242, 300, (326)	$Y = 5.97E^{+05}X - 3.60E^{+06}$	0.9967	0.01 – 300	0.068	0.226	1.04	3.54
(−)-epicatechin	8	21.81	240, (280)	$Y = 2.05E^{+05}X - 5.81E^{+05}$	0.9916	0.01 – 300	0.044	0.144	0.56	2.65
Syringic acid	9	27.61	(276)	$Y = 1.38E^{+06}X - 5.21E^{+06}$	0.9999	0.05 – 200	0.024	0.080	0.25	1.33
Ferulic acid	10	4.27	233, 296, (324)	$Y = 7.72E^{+05}X + 4.44E^{+05}$	0.9937	0.01 – 500	0.060	0.200	0.28	1.08
Rutin	11	4.68	(236), 260, 360	$Y = 2.72E^{+05}X - 1.57E^{+06}$	0.9978	0.01 – 300	0.068	0.226	0.58	2.99
Isoquercetin	12	10.01	255, (352)	$Y = 3.18E^{+05}X + 1.46E^{+05}$	0.9923	0.01 – 300	0.040	0.130	0.87	2.25
Cinnamic acid	13	14.21	(278)	$Y = 2.22E^{+06}X + 2.40E^{+06}$	0.9921	0.05 – 500	0.042	0.142	0.35	1.07

SD; standard deviation, LOD, limit of detection; LOQ; limit of quantification.
[a] *X* is the peak area and Y is the concentration (μg/mL) of the compound.
[b] LOD was calculated based on a signal-to-noise ratio of 3:1 (n = 10).
[c] LOQ was calculated based on a signal-to-noise ratio of 10:1 (n = 10).
[d] RSD (%) = ((standard deviation/mean) × 100) (n = 10).

Table 5
Mean content (mg/100 g dw) of phenolic compounds of brewer's spent yeast extracts in free and bound fractions.

Compound	Peak no.	Free (mg/100 g dw)	Bound (mg/100 g dw)	∑ TPC (mg/100 g dw)[a]	Free/∑ TPC (%)[b]	Bound/∑ TFC (%)[b]
Gallic acid	1	21.3 ± 3.5	2.11 ± 0.51	23.4	38	3
Protocatechuic acid	2	Nq (0.250)	13.1 ± 1.30	13.1	–	22
(±)-Catechin	3	34.2 ± 5.8	24.6 ± 4.11	58.8	62	41
p-Coumaric acid	4	Nq (0.274)	10.3 ± 1.01	10.3	–	17
Ferulic acid	10	Nq (0.200)	9.22 ± 0.50	9.20	–	15
Cinnamic acid	13	Nq (0.142)	1.24 ± 0.00	1.21	–	2
∑ TPC		55.5 ± 9.3	60.6 ± 7.40	116	–	–

Data are expressed as mean values ± standard deviation of six independent experiments, analysed in duplicate (n = 12).
Nq: not quantified, concentrations <LOQ (values represented in brackets in mg/100 g).
(–): not calculate.
[a] Total phenolic content (TFC) calculated as sum of individual phenolic compounds from free and bound fractions.
[b] Calculated considering the ∑ TPC of the free/bound phenolic fraction.

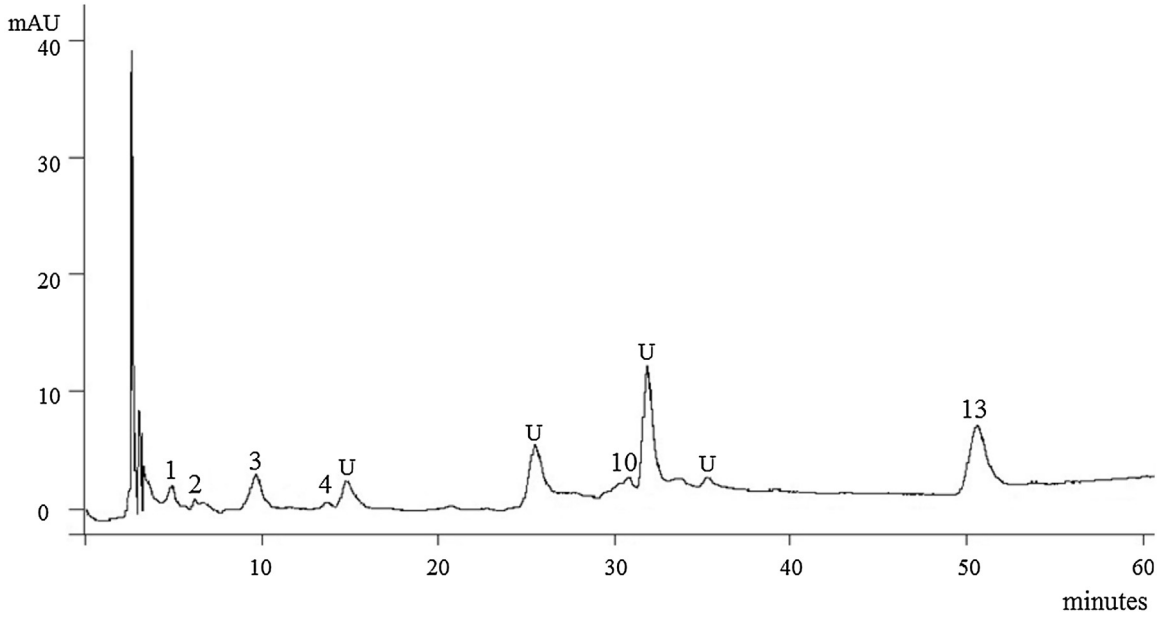

Fig. 1. Typical HPLC-PDA (detection at 280 nm) chromatogram of bound phenolic compounds fraction from brewer's spent yeast extract. Peaks identification: (1) gallic acid; (2) protocatechuic acid; (3) (±)-catechin; (4) p-coumaric acid; (10) ferulic acid and (13) cinnamic acid. U- unknown peaks.

Conflict of interest

The authors declare that there is no conflict of interest.

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